FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 10806-155

U.S. APPLIC. NO. (if known, see 37 CFR 1.5): 09/743023

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE99/01222	5 July 1999	8 July 1998

TITLE OF INVENTION

METHOD FOR THE PRODUCTION OF RECOMBINANT PEPTIDES WITH A LOW AMOUNT OF TRISULFIDES

APPLICANT(S) FOR DO/EO/US

HEMMENDORFF, Barbro; CASTAN, Andreas; PERSSON, Anders

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] have been transmitted by the International Bureau.
   c. [] have not been made; however, the time limit for making such amendment has NOT expired.
   d. [X] have not been made and will not be made.

8. [] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

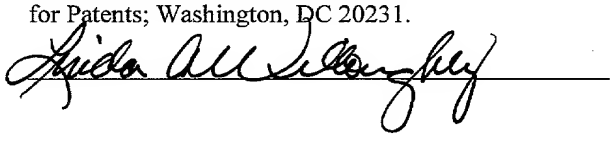
13. [X] A FIRST preliminary amendment.
    [] A SECOND or SUBSEQUENT preliminary amendment.

14. [] A substitute specification.

15. [] A change of power of attorney and/or address letter.

16. [X] Other items or information: Copy of published International Application No. WO 00/02900, including International Search Report

CERTIFICATE OF EXPRESS MAILING
"Express Mail" mailing label #: EL378867410US
Date of Deposit: January 4, 2001
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Box PCT; Assistant Commissioner for Patents; Washington, DC 20231.



525 Rec'd PCT/PTO 04 JAN 2001

U.S. APPLIC. NO. (if known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/743023	PCT/SE99/01222	10806-155

	CALCULATIONS	PTO USE ONLY
17. The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
[] Search Report has been prepared by the EPO or JPO $860.00		
[] International preliminary examination fee paid to USPTO (37 CFR 1.482) $690.00		
[] No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
[X] Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
[] International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1000.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	18 -20 =	0	x $18.00	$	
Independent Claims	2 -3 =	0	x $80.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	

TOTAL OF ABOVE CALCULATIONS =	$ 1130.00	
[] Applicant(s) claim(s) small entity status, 37 C.F.R. 1.27. The fees indicated above are reduced by 1/2.	$	
SUBTOTAL =	$ 1130.00	
Processing fee of $130.00 for furnishing the English translation later than the [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$ 1130.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$	
TOTAL FEES ENCLOSED =	$ 1130.00	
	Amount to be refunded	$
	Amount to be charged	$

a. [X] A check in the amount of $1130.00 to cover the above fees is enclosed.

b. [] Please charge my Deposit Account No. in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-1133.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

☒ CUSTOMER NO. 24256 OR

DINSMORE & SHOHL
1900 Chemed Center
255 East Fifth Street
Cincinnati, Ohio 45202
(513) 977-8200

SIGNATURE

30,468
REGISTRATION NUMBER

HOLLY D. KOZLOWSKI
TYPED OR PRINTED NAME

4 JANUARY 2001
DATE

BASED ON FORM PTO-1390 (Rev. 10-2000)

636181.01

Docket No. 10806-155
PATENT

CERTIFICATE OF MAILING
"Express Mail" mailing label #: EL378867410US
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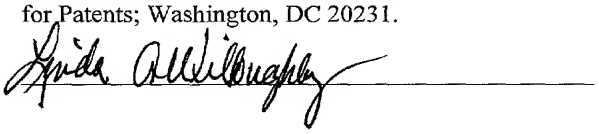

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Barbro Hemmendorff et al	:	Paper No.:
Based on:	PCT/SE99/01222	:	Group Art Unit:
Filing Date:	January 4, 2001	:	Examiner:

For: **Method for the Production of Recombinant Peptides with a Low Amount of Trisulfides**

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, DC 20231

Dear Sir:

Prior to calculation of the filing fee and first action by the Examiner, please amend the present application as follows:

In the Claims:

Please cancel claims 4, 9 and 10.

Please amend claims 1-3 and 5-8 as follows:

1. (Amended) Method for the production of recombinant peptides with a low amount of trisulfides, [characterized by the addition of] comprising fermenting cells to produce the recombinant peptides, wherein a metal of salt is added during or after the fermentation step.

2. (Amended) Method for the reduction of the amount of trisulfides in the production of recombinant peptides, [characterized by the addition of] comprising fermenting cells to produce recombinant peptides, wherein a metal salt is added during or after fermentation.

3. (Amended) Method according to claim 1, wherein [any of claims 1 to 2 in which] the addition is performed directly after fermentation.

5. (Amended) Method according to claim 1, wherein [any of claims 1 to 4 in which] pH is equal to or lower than pH 7.

6. (Amended) Method according to claim 1, wherein [any of claims 1 to 5 in which] the metal salt [preferably] is potassium or sodium salt.

Claim 7, line 1, delete "preferably".

8. (Amended) Method according to claim 1, wherein [any of claims 1 to 7 in which] the peptide [preferably] is growth hormone [and more preferably human growth hormone].

Please add the following claims 11-19:

--11. (NEW) Method according to claim 1, wherein the metal salt is an alkali metal salt or an alkali earth metal salt.--

--12. (NEW) Method according to claim 1, wherein the peptide is human growth hormone.--

--13. (NEW) Method according to claim 2, wherein the addition is performed directly after fermentation.--

--14. (NEW) Method according to claim 2, wherein the metal salt is an alkali metal salt or an alkali earth metal salt.--

--15. (NEW) Method according to claim 2, wherein pH is equal to or lower than pH 7.--

--16. (NEW) Method according to claim 2, wherein the metal salt is potassium or sodium salt.--

--17. (NEW) Method according to claim 16 in which the salt is potassium- or sodium phosphate or acetate.--

--18. (NEW) Method according to claim 2, wherein the peptide is growth hormone.--

--19. (NEW) Method according to claim 2, wherein the peptide is human growth hormone.--

REMARKS

By the present Amendment, the claims are amended to omit their multiple dependency and for matters of form in accordance with customary U.S. patent practice. Support for claims 11 and 14 may be found in original claim 4, support for claims 12, 18 and 19 may be found in original claim 8 and support for claims 13 and 15-17 may be found in original claims 3 and 5-7, respectively. Since these changes do not involve any introduction of new matter, entry is believed to be in order and is respectfully requested.

Respectfully submitted,

Holly D. Kozlowski, Reg. No. 30,468
Dinsmore & Shohl LLP
1900 Chemed Center
255 East Fifth Street
Cincinnati, Ohio 45202
(513) 977-8568

636137 01

Method for the production of recombinant peptides with a low amount of trisulfides.

The invention relates to a method for the production of recombinant peptides with a low amount of trisulfides which is characterized by the addition of a metal salt during or after the fermentation step and to a method for reduction of the amount of trisulfides in the production of recombinant peptides, characterized by the addition of a metal salt during or after fermentation. The peptide is preferably human growth hormone and the salt preferably a potassium or sodium phosphate.

Background

In the recombinant production of peptides, especially in the production of pharmaceuticals, the amount of contamination, such as variants of the wanted protein, should be reduced as much as possible both from economical and therapeutical aspects.

In the recombinant production of peptides, variants with an extra sulfur atom in a disulfide bridge sometimes are found, and the present invention relates to this problem.

Human Growth hormone, hGH, is a protein consisting of a single chain of 191 amino acids. The molecule is cross-linked by two disulfide bridges and the monomeric form has a molecular weight of 22 kDa.

hGH preparations have been prepared from human pituitaries, but nowadays the products on the market are produced by recombinant methods, rhGH.

Two types of therapeutically useful recombinant hGH preparations are present on the market: the authentic one, e.g. Genotropin®, Pharmacia & Upjohn AB, and an analogue with an additional methionine residue at the N-terminal end, e.g. Somatonorm®.

hGH is used to stimulate linear growth in patients with hypo pituitary dwarfism or Turner's syndrome but other indications have also been suggested.

A new variant of human growth hormone, hGH, has been found and reference is given to Pavlu et al, 1993, Bioseparation 3, 257-265. This variant has been identified and characterized, see Andersson et al, 1996, Int. J. Peptide, Protein,. Res. 47, 311-321. The

variant, which is formed during the expression of hGH in Escherichia coli, is found to be more hydrophobic than rhGH and has been structurally defined as a trisulfide variant of rhGH. The variant is only formed during synthesis in E Coli and has not been found in hGH preparations from human pituitaries.

This phenomenon of the trisulfides in peptides, produced by recombinant methods, has also been described for recombinant superoxide dismutase (Briggs et al, 1987, Biochem., Biophys. Acta, 537, 100-109) and for a mutein of interleukin, (Breton J et al. J. Chromatogr. A., 1995, 709(1), 135-46).

In WO 94/24127 a method for converting a hydrophobic derivative of a growth hormone into the native form of growth hormone is disclosed. The hydrophobic derivative of the growth hormone comprises an extra sulfur atom. The method is a chemical treatment of the derivative of growth hormone with a mercapto compound. As examples are cystein, gluthatione, 2-mercapto ethanol and dithiothreitol given.

In WO 96/02570 a method is disclosed comprising the chemical treatment with a sulfite compound for the conversion of the derivative of growth hormone into the native form. Mercapto compounds and sulfite compounds are used in the redox-reaction for the conversion of the already formed growth hormone comprising an extra sulfur atom.

The invention

We have now found a new method for the reduction of the amount of trisulfides in the production of recombinant peptides, e.g. both proteins and smaller peptides.

The invention is based on the novel and unexpected finding that the amount of trisulfides in the production of recombinant peptides can be reduced by the addition of a metal salt, preferably in excess, already during or after fermentation and not, as earlier suggested, by conversion of the formed trisulfide of growth hormone into the native form.

This reduced amount of the derivative is due to inhibition of the activity of H_2S in the medium and the prevention of the formation of the modified growth hormone comprising an extra sulfur atom

The addition can be done directly after fermentation, e.g. after the fermentation has been terminated and the cells are harvested and before further process steps.

The addition can e.g. be done with a buffer including the salt.

The protein can be any recombinant protein but is preferably recombinant growth hormone which can be both human and animal such as human growth hormone (hGH), bovine growth hormone (bGH) and porcine growth hormone (pGH).

The metal salt can be any metal chosen among alkalimetal and earth metal.

pH is preferably equal to or lower than pH 7. More preferable pH is equal to or lower than 6.8 and most preferable pH is equal to or lower than 6.0.

The pH regulation can be achieved with a selected buffer including the metal salt.

The metal is preferably alkali, such as sodium or potassium and the salt is preferably sodium or potassium phosphate or acetate.

The concentration of free sulfide ions is minimized by addition of the metal salt in molar excess.

The used metal salt is preferably not a sulfite or a mercapto compound.

The attached claims define the invention.

Figure 1 shows the amount of trisulfide-GH in the extracts.

Figure 2 shows the induction and inhibition of trisulfide formation in GH

In the examples below a recombinant produced hGH has been produced or used, but the invention as claimed is not limited to this peptide. The trisulfide variant is named trisulfide-GH.

EXAMPLES

hGH was produced in *E. Coli* according to known methods. Reference can be given to EP 177343, example 8.

The transformant of *E. Coli* was fermented in the medium, the culture was agitated under aeration and glucose was added. The fermentation was terminated by turning off the glucose and aeration. At this point a reference sample was taken. Thereafter the cells were harvested.

For the production of pure hGH, the harvested cells were concentrated, washed, solubilized by freezing, thawed and purified according to known methods.

Example 1. pH variation, lab scale.

The culture was harvested and the cells were concentrated by microfiltration. The pH was 7.3 in the cell concentrate. Four batches of the cell concentrate were taken. In three batches (500 ml) the pH was adjusted to 6.5, 7.0 and 7.8, with HCl or NaOH, respectively. The fourth batch is the non-treated comparison sample. Thereafter the cell concentrates were frozen.

The four batches were thawed and the cell concentrates were diluted twice with a buffer containing 10 mM Tris-HCl and 1 mM Na_2-EDTA pH 8.2. Cell free extracts were obtained by centrifugation.

The amount of trisulfide-GH in the extracts was determined.

The result is shown in Figure 1.

It was found that the amount of trisulfide-GH was highest at pH 7.8 (12%). This could be compared to the fourth batch which was not pH-changed.

A pH above 7.0 gave too high amount of trisulfide-GH in this experiment, thus pH should be lower.

Example 2. Pilot scale

The culture was harvested and the cells were concentrated by microfiltration. The pH in the cell concentrate was 7.2. The cell concentrate was divided in two portions (about 30 L).

Cell concentrate A was washed with about one volume of water and was thereafter frozen at - 30°C.

Cell concentrate B was washed with about one volume of 0.05 M potassium phosphate buffer, pH 6.6. The pH in cell concentrate B was 6.8. The cell concentrate was thereafter frozen at-30°C.

After thawing, the concentrated cells were extracted by diafiltration with Tris-HCl /EDTA buffer and the amount of trisulfides was determined. The amount of trisulfide-GH was 6% in extract A and about 3 % in extract B, thus the double in A compared to B. This showed that low pH and the metal salt buffer reduces the amount of the trisulfide variant of growth hormone.

Example 3. Pilot scale

The amount of trisulfides in the reference sample, taken before harvest, was determined. The culture was harvested and the cells were concentrated by microfiltration. The pH in the cell concentrate was 7.2. The cell concentrate was divided in two portions (about 30 L). Cell concentrate C was washed with about one volume of water and was thereafter frozen at - 30°C.

Cell concentrate D was washed with about one volume of 0.9 % NaCl in water. The pH in that cell concentrate was 7.2. The cell concentrate was thereafter frozen at-30°C.

After thawing, the concentrated cells were extracted by diafiltration with Tris-HCl /EDTA buffer and the amount of trisulfides was determined. The amount of trisulfide-GH was about 5 % in extract C and about 4.8 % in D, thus the same in C and D. The ratio of trisulfide-GH in extract C : reference sample was 5.0 % : 2.0 % = 2.5 and the ratio of trisulfide-GH in extract D : reference sample was 4.7 % : 2.0 % = 2.4

This showed that for a periplasmatic extract not only the addition of a metal salt but also the low pH is of importance.

Example 4. Pilot scale

The amount of trisulfides in the reference sample, taken before harvest, was determined. The culture was harvested and the cells were concentrated by microfiltration. The pH in the cell concentrate was 7.2. The cell concentrate (E) was washed with about one volume of 0.025 M sodium phosphate buffer pH 6.0, to which 1 ml/L HCl 37 % was added. The pH in cell concentrate E was 5.9. The cell concentrate was thereafter frozen at-30°C.

After thawing the concentrated cells were extracted by diafiltration with Tris-HCl /EDTA buffer and the amount of trisulfides was determined.

The ratio of trisulfide-GH in extract E : reference sample was 1.6 % : 1.4 % = 1.1.

This showed that the amount of trisulfide-GH can be reduced by the addition of a metal salt and a low pH.

Example 5. Pilot scale

The amount of trisulfides in the reference sample, taken before harvest, was determined. The culture was harvested and the cells were concentrated by microfiltration. The pH in the cell concentrate was 7.2. The cell concentrate was divided in two portions (about 30 L).

Cell concentrate F was washed with about one volume of acetate buffer, containing sodium acetate x $3H_2O$, 8.03 g/L and acetic acid (100 %) 2.35 ml/L. The pH in cell concentrate F was 5.9.The cell concentrate was thereafter frozen at - 30°C.

Cell concentrate G was washed with about one volume of 0.025 M sodium phosphate buffer pH 6.0, to which 0.5 ml/L concentrated H_2SO_4 was added. The pH in cell concentrate G was 5.9. The cell concentrate was thereafter frozen at-30°C.

After thawing the concentrated cells were extracted by diafiltration with Tris-HCl /EDTA buffer and the amount of trisulfides was determined.

The ratio of trisulfide-GH in extract F : reference sample was 3.4 % : 3.1 % = 1.1 and the ratio of trisulfide-GH in extract G : reference sample was 2.6 % : 3.1 % = 0.8.

This showed that the amount of trisulfide-GH can be reduced by the addition of a metal salt and a low pH.

Example 6. Comparison of buffers and pH.

250µl of pure hGH (from the production of Genotropin®) in water (2.436 mg/ml) + 250µl of different 100 mM buffers , see Table 1, were mixed. Saturated H_2S (0.11 M) in distilled water was used immediately after preparation. 50µl of distilled water (control) or H_2S in three different dilutions was added to each sample. (0.5, 0.1 and 0.02 mM H_2S, respectively)

The concentration was thereafter 1.11 mg hGH/ml.

These solutions were incubated with the different concentrations of H_2S during 3 hours at room temperature for the preparation of the trisulfide variant of hGH.

After incubation, freezing, thawing and desalting of all samples in 25 mM Tris-HCl at pH 7.6, the amount of trisulfide was analyzed.

The buffers were prepared according to standard tables.

Table 1

Na-phosphate, pH 7.8

Na-phosphate, pH 7.0

Na-phosphate, pH 6.5

Na-phosphate, pH 6.0

Na-citrate, pH 6.2

Tris-HCl, pH 7.6

Ammonium citrate, pH 6.2

The result is shown in Figure 2.

Ammonium citrate gave no reduction of trisulfides despite the low pH.

Na-phosphate at pH 6.0 gave the best result but also Na-phosphate at higher pH can be used.

This showed that for pure hGH the addition of a metal salt is of importance for the amount of trisulfides.

CLAIMS

1. Method for the production of recombinant peptides with a low amount of trisulfides, characterized by the addition of a metal salt during or after the fermentation step.

2. Method for the reduction of the amount of trisulfides in the production of recombinant peptides, characterized by the addition of a metal salt during or after fermentation.

3. Method according to any of claims 1 to 2 in which the addition is performed directly after fermentation.

4. Method according to any of claims 1 to 3 in which the metal salt is chosen among alkali metals and earth metals.

5. Method according to any of claims 1 to 4 in which pH is equal to or lower than pH 7.

6. Method according to any of claims 1 to 5 in which the metal preferably is potassium or sodium.

7. Method according to claim 6 in which the salt preferably is potassium- or sodium phosphate or acetate.

8. Method according to any of claims 1 to 7 in which the peptide preferably is growth hormone and more preferably human growth hormone.

9. Use of a metal salt in the production of recombinant peptides during or after the fermentation step for the reduction of the amount of trisulfides in the recombinant product.

10. Use of metal salt for the reduction of the amount of trisulfides in the production of recombinant peptides by the addition of a metal salt during or after fermentation

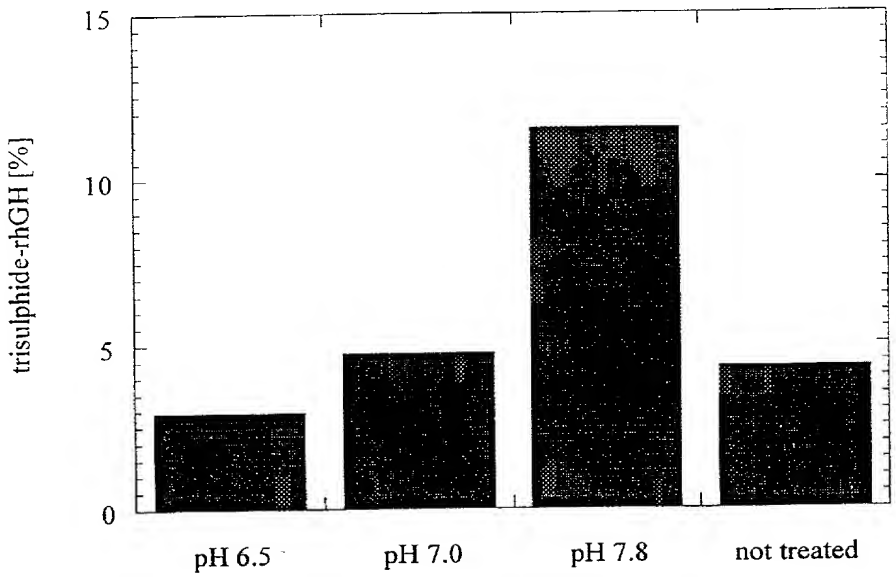

Figure 1

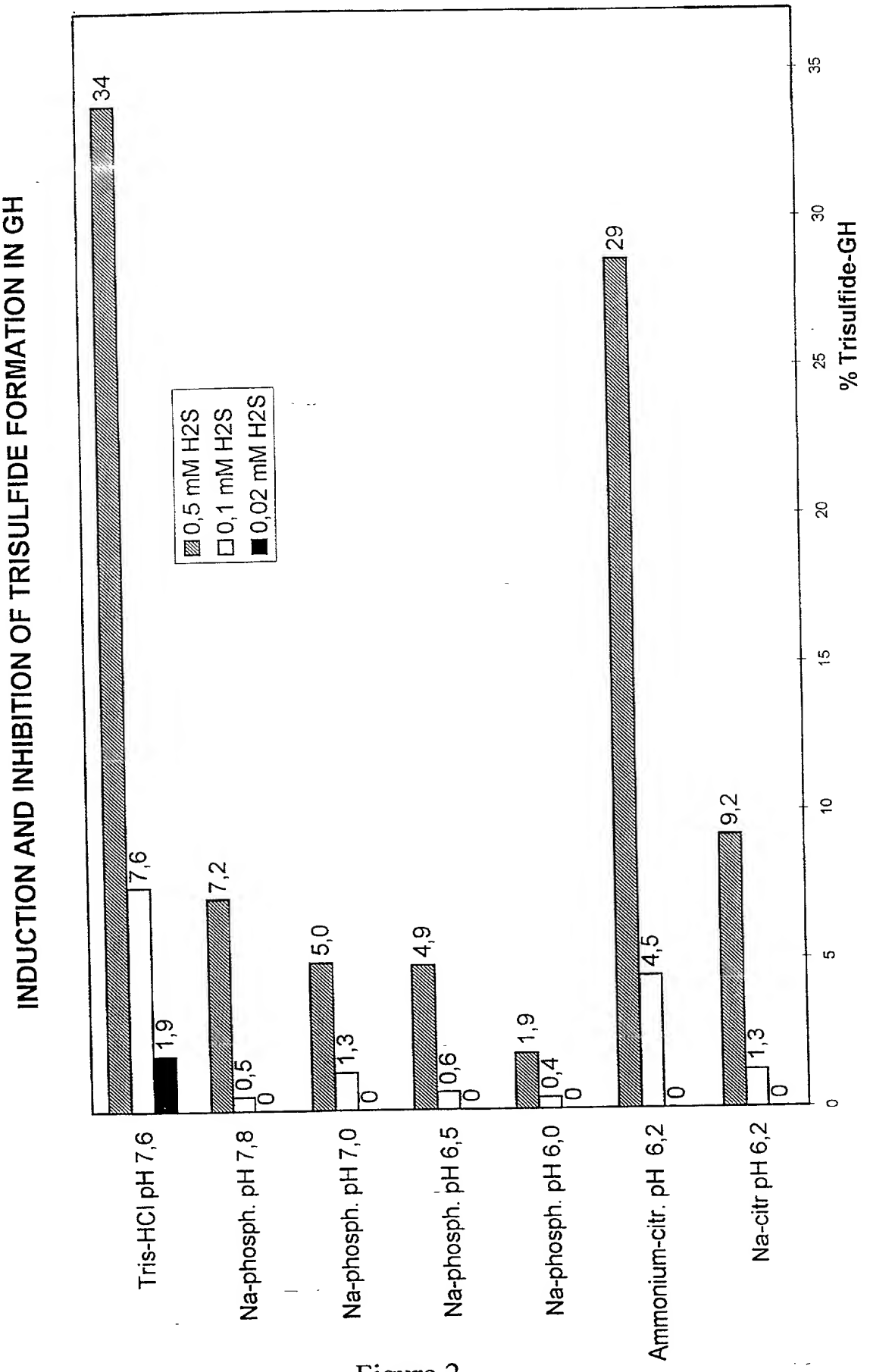
INDUCTION AND INHIBITION OF TRISULFIDE FORMATION IN GH
0,5 mM H2S
0,1 mM H2S
0,02 mM H2S
% Trisulfide-GH
0
5
10
15
20
25
30
35
34
7,6
1,9
7,2
0,5
0
5,0
1,3
0
4,9
0,6
0
1,9
0,4
0
29
4,5
0
9,2
1,3
0
Tris-HCl pH 7,6
Na-phosph. pH 7,8
Na-phosph. pH 7,0
Na-phosph. pH 6,5
Na-phosph. pH 6,0
Ammonium-citr. pH 6,2
Na-citr pH 6,2

Figure 2

1830 US

DECLARATION
and
POWER OF ATTORNEY

U.S. NATIONAL PHASE OF INTERNATIONAL APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **Method for the Production of Recombinant Peptides with a Low Amount of Trisulfides**, the specification of which was filed as International Application No. PCT/SE99/01222 on July 5, 1999,

[ ] and was amended under Article 19 on _____ (if applicable).

[ ] and was amended under Article 34 on _____ (if applicable).

[X] and was assigned U.S. Application Serial No. 09/743,023 and was amended on January 4, 2001.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits and/or U.S. Provisional application priority benefits under Title 35, United States Code, §119 of any foreign application(s) or U.S. Provisional applications for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign and U.S. Provisional Application(s)				
			Priority Claimed	
Number	Country	Day/Month/Year Filed	Yes	No
9802454-0	Sweden	8 July 1998	X	

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material

information as defined in Title 37, Code of Federal Regulation, §1.56(a) which occurred between the filing date of the prior application and the PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby appoint Holly D. Kozlowski, Registration No. 30,468; Ronald J. Snyder, Registration No. 31,062; James D. Liles, Registration No. 28,320; Lynda E. Roesch, Registration No. 29,696; Martin J. Miller, Registration No. 35,953; John V. Harmeyer, Registration No. 41,815; Scott N. Barker, Registration No. 42,292; Geoffrey L. Oberhaus, Registration No. 42,955; Joseph P. Mehrle, Registration No. 45,535; John P. Colbert, Registration No. 45,765; Stephen S. Wentsler, Registration No. 46,403; and Ryan O. White, Registration No. 45,541, my attorneys, c/o Dinsmore & Shohl, 1900 Chemed Center, 255 East Fifth Street, Cincinnati, Ohio 45202 (513) 977-8200, with full power in each of them, of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

12-

The undersigned hereby authorizes the above-named U.S. attorneys to accept and follow instructions from **Pharmacia AB** as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the undersigned and the aforenamed U.S. attorneys. In the event of a change in the firm or persons from whom instructions may be taken, the aforenamed U.S. attorneys will be so notified in writing by the undersigned.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

****

1-00

Full name of sole or first inventor: Barbro Hemmendorff

Inventor's signature Barbro Hemmendorff 2001-02-20
Date

Residence: Drejarvägen 8, SE-141 73 Huddinge, Sweden SE X
Citizenship: Sweden
Post Office Address: Drejarvägen 8, SE-141 73 Huddinge, Sweden

2-00 Full name of second inventor: Andreas Castan

Inventor's signature ______________________ 2001-02-26
Date

Residence: Ivar Vidfamnesgatan 2, SE-126 52 Hägersten, Sweden SEX
Citizenship: Germany
Post Office Address: Ivar Vidfamnesgatan 2, SE-126 52 Hägersten, Sweden

3-00 Full name of third inventor: Anders Persson

Inventor's signature ______________________ 2001-02-21
Date

Residence: Kålsängsgränd 6 D, SE-753 19 Uppsala, Sweden SEX
Citizenship: Sweden
Post Office Address: Kålsängsgränd 6 D, SE-753 19 Uppsala, Sweden

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